CLAIMS

1. A method of identifying a melanoma comprising the steps of

   a. obtaining a tissue sample; and

   b. measuring the expression levels in the sample of genes encoding mRNA
   corresponding to

   PLAB (SEQ ID NO: 1) and L1CAM (SEQ ID NO: 2); or

   PLAB, L1CAM andNTRK3 (SEQ ID NO: 3)

   wherein the gene expression levels above pre-determined cut-off levels are indicative
   of the presence of a melanoma in the sample.

2. The method of claim 1 further comprising measuring the expression level of a gene
encoding tyrosinase (SEQ ID NO: 999).

3. The method of claim 1 further comprising measuring the expression level of a gene
constitutively expressed in the sample.

4. The method of claim 3 wherein the gene encodes PBGD (SEQ ID NOs: 979).

5. The method of claim 1 further comprising measuring the expression levels of at least
one gene encoding an mRNA corresponding to a psid selected from the group consisting
of SEQ ID NOs: 29-978.

6. The method of claim 1, 2, 3, 4 or 5 wherein the sample is obtained from a lymph node.

7. The method of claim 6 wherein the lymph node is a sentinel lymph node.

8. The method of claim 1, 2, 3, 4 or 5 wherein the sample is obtained from a biopsy.

9. The method of claim 1, 2, 3, 4 or 5 wherein the method is performed intra-operatively.

10. The method of claim 1, 2, 3, 4 or 5 wherein the melanoma is a micrometastasis.

11. The method of claim 1, 2, 3, 4 or 5 wherein the specificity and sensitivity are
sufficient to detect metastasis of melanoma.

12. The method of claim 11, wherein the specificity is at least 95% based on a
comparison of hematoxylin and eosin (H&E) and immunohistochemical (IHC) negative
nodes.

13. The method of claim 11 wherein the specificity is at least 97% based on a
comparison of H&E and IHC negative nodes.

14. The method of claim 11 wherein the specificity is at least 99% based on a
comparison of H&E and IHC negative nodes.

15.   The method of claim 11 wherein the sensitivity is at least at least 80% based on a comparison of hematoxylin and eosin (H&E) and immunohistochemical (IHC) positive nodes.

16.   The method of claim 11 wherein the sensitivity is at least 85% based on a comparison of H&E and IHC positive nodes.

17.   The method of claim 11 wherein the sensitivity is at least 90% based on a comparison of H&E and IHC positive nodes.

18.   The method of claim 11 wherein the specificity and sensitivity are at least 97% based on a comparison of H&E and IHC negative nodes and at least 85% based on a comparison of H&E and IHC positive nodes, respectively.

19.   The method of claim 1, 2, 3, 4 or 5 wherein the pre-determined cut-off levels are at least two-fold over-expression in tissue having metastatic melanoma relative to benign melanocyte or normal tissue.

20.   The method of claim 1, 2, 3, 4 or 5 wherein gene expression is measured on a microarray or gene chip.

21.   The method of claim 1, 2, 3, 4 or 5 wherein gene expression is determined by nucleic acid amplification conducted by polymerase chain reaction (PCR) of RNA extracted from the sample.

22.   The method of claim 21 wherein the PCR products comprise at least one of SEQ ID NOs: 25-28.

23.   The method of claim 22 wherein the PCR products include fluorophores.

24.   The method of claim 23 wherein the fluorophores are selected from the group consisting of Fam, Texas Red, Cal Red, Cl, Cy5, and Cy3.

25.   The method of claim 24 wherein the fluorophores correspond to PLAB: Fam; LlCAM: Texas Red or Cal Red, tyrosinase: Cl; PBGD: Cy5, where applicable.

26.   The method of claim 21 wherein said PCR is reverse transcription polymerase chain reaction (RT-PCR).

27.   The method of claim 26, wherein the RT-PCR further comprises one or more internal control reagents.

28.   The method of claim 21 wherein RNA is extracted from the sample by:

    a.   homogenizing the sample to produce an homogenate;

b. contacting the homogenate with a substrate containing, or to which is affixed, an
RNA-binding material;

c. allowing the RNA to bind to the RNA binding material;

d. washing the substrate under conditions sufficient to remove any contaminants,
interferents and un-bound RNA; and

e. eluting bound RNA from the substrate.

29.   The method of claim 1, 2, 3, 4 or 5 further comprising reducing melanin in the
sample.

30.   The method of claim 29 wherein melanin concentration is reduced by homogenizing
the sample to produce an homogenate and passing the homogenate through a matrix to
which melanin adheres, bonds, or is affixed.

31.   The method of claim 30 wherein said matrix comprises polymeric beads.

32.   The method of claim 30 wherein said matrix comprises silica.

33.   The method of claim 28 wherein the RNA is extracted in less than about 8 minutes.

34.   The method of claim 28 wherein the RNA is extracted in less than about 6 minutes.

35.   The method of claim 1, 2, 3, 4 or 5 wherein gene expression is measured by
measuring the protein encoded by the gene.

36.   The method of claim 35 wherein the protein is detected by an antibody specific to
the protein.

37.   A method of identifying a melanoma comprising the steps of

a. obtaining a tissue sample; and

b. measuring the expression levels in the sample of genes encoding niRNA
recognized by the primer/probe sets selected from the group consisting of

SEQ ID NOs: 4-6 or SEQ ID NOs: 7-9 and SEQ ID NOs: 10-12 or SEQ ID NOs: 13-
15; or

SEQ ID NOs: 4-6 or SEQ ID NOs: 7-9 and SEQ ID NOs: 10-12 or SEQ ID NOs: 13-
15 and SEQ ID NOs: 16-18

wherein the gene expression levels above pre-determined cut-off levels are indicative
of the presence of a melanoma in the sample.

38.   The method of claim 37 further comprising measuring the expression level of a gene
encoding tyrosinase (SEQ ID NO: 999).

39.   The method of claim 37 further comprising measuring the expression level of a gene constitutively expressed in the sample.

40.   The method of claim 39 wherein the gene encodes PBGD (SEQ ID NO: 979).

41.   The method of claim 37 further comprising measuring the expression levels of at least one gene encoding an mRNA corresponding to a psid selected from the group consisting of SEQ ID NOs: 29-978.

42.   The method of claim 37, 38, 39, 40 or 41 wherein the sample is obtained from a lymph node.

43.   The method of claim 42 wherein the lymph node is a sentinel lymph node.

44.   The method of claim 37, 38, 39, 40 or 41 wherein the sample is obtained from a biopsy.

45.   The method of claim 37, 38, 39, 40 or 41 wherein the method is performed intra-operatively.

46.   The method of claim 37, 38, 39, 40 or 41 wherein the melanoma is a micrometastasis.

47.   The method of claim 37, 38, 39, 40 or 41 wherein the specificity and sensitivity are sufficient to detect metastasis of melanoma.

48.   The method of claim 47, wherein the specificity is at least 95% based on a comparison of hematoxylin and eosin (H&E) and immunohistochemical (IHC) negative nodes.

49.   The method of claim 47 wherein the specificity is at least 97% based on a comparison of H&E and IHC negative nodes.

50.   The method of claim 47 wherein the specificity is at least 99% based on a comparison of H&E and IHC negative nodes.

51.   The method of claim 47 wherein the sensitivity is at least at least 80% based on a comparison of hematoxylin and eosin (H&E) and immunohistochemical (IHC) positive nodes.

52.   The method of claim 47 wherein the sensitivity is at least 85% based on a comparison of H&E and IHC positive nodes.

53.   The method of claim 47 wherein the sensitivity is at least 90% based on a comparison of H&E and IHC positive nodes.

54.   The method of claim 47 wherein the specificity and sensitivity are at least 97% based on a comparison of H&E and IHC negative nodes and at least 85% based on a comparison of H&E and IHC positive nodes, respectively.

55.   The method of claim 37, 38, 39, 40 or 4 1 wherein the pre-determined cut-off levels are at least two-fold over-expression in tissue having metastatic melanoma relative to benign melanocyte or normal tissue.

56.   The method of claim 37, 38, 39, 40 or 4 1 wherein gene expression is measured on a microarray or gene chip.

57.   The method of claim 37, 38, 39, 40 or 4 1 wherein gene expression is determined by nucleic acid amplification conducted by polymerase chain reaction (PCR) of RNA extracted from the sample.

58.   The method of claim 57 wherein the PCR products comprise at least one of SEQ ID NOs: 25-28.

59.   The method of claim 57 wherein the PCR products include fluorophores.

60.   The method of claim 59 wherein the fluorophores are selected from the group consisting of Fam, Texas Red, Cal Red, Cl, Cy5 and Cy3.

6 1.   The method of claim 60 wherein the fluorophores correspond to PLAB : Fam; LlCAM: Texas Red or Cal Red, tyrosinase: Cl; PBGD: Cy5, where applicable.

62.   The method of claim 57 wherein said PCR is reverse transcription polymerase chain reaction (RT-PCR).

63.   The method of claim 62, wherein the RT-PCR further comprises one or more internal control reagents.

64.   The method of claim 57 wherein RNA is extracted from the sample by:

    a.   homogenizing the sample to produce an homogenate;

    b.   contacting the homogenate with a substrate containing, or to which is affixed, an RNA-binding material;

    c.   allowing the RNA to bind to the RNA binding material;

    d.   washing the substrate under conditions sufficient to remove any contaminants, interferents and un-bound RNA; and

    e.   eluting bound RNA from the substrate.

65.   The method of claim 37, 38, 39, 40 or 4 1 further comprising reducing melanin in the sample.

66.   The method of claim 65 wherein melanin concentration is reduced by homogenizing the sample to produce an homogenate and passing the homogenate through a matrix to which melanin adheres, bonds, or is affixed.

67.   The method of claim 66 wherein the matrix comprises polymeric beads.

68.   The method of claim 66 wherein the matrix comprises silica.

69.   The method of claim 64 wherein the RNA is extracted in less than about 8 minutes.

70.   The method of claim 64 wherein the RNA is extracted in less than about 6 minutes.

71.   The method of claim 37, 38, 39, 40 or 41 wherein gene expression is measured by measuring the protein encoded by the gene.

72.   The method of claim 71 wherein the protein is detected by an antibody specific to the protein.

73.   A method of distinguishing a malignant melanocyte from a benign melanocyte comprising the steps of

    a.   obtaining a tissue sample; and

    b.   measuring the expression levels in the sample of genes encoding

    PLAB (SEQ ID NO: 1) and L1CAM (SEQ ID NO: 2); or

    PLAB, L1CAM and NTRK3 (SEQ ID NO: 3)

    wherein the gene expression levels above pre-determined cut-off levels are indicative of the presence of a malignant melanocyte in the sample.

74.   The method of claim 73 further comprising measuring the expression level of a gene encoding tyrosinase (SEQ ID NO: 999).

75.   The method of claim 73 further comprising measuring the expression level of a gene constitutively expressed in the sample.

76.   The method of claim 75 wherein the gene encodes PBGD (SEQ ID NO: 979).

77.   The method of claim 73 further comprising measuring the expression levels of at least one gene encoding an mRNA corresponding to a psid selected from the group consisting of SEQ ID NOs: 29-978.

78.   The method of claim 73, 74, 75, 76 or 77 wherein the sample is obtained from a lymph node.

79.   The method of claim 78 wherein the lymph node is a sentinel lymph node.

80.   The method of claim 73, 74, 75, 76 or 77 wherein the sample is obtained from a biopsy.

81. The method of claim 73, 74, 75, 76 or 77 wherein the method is performed intra-operatively.

82. The method of claim 73, 74, 75, 76 or 77 wherein the melanoma is a micrometastasis.

83. The method of claim 73, 74, 75, 76 or 77 wherein the specificity and sensitivity are sufficient to detect metastasis of melanoma.

84. The method of claim 83, wherein the specificity is at least 95% based on a comparison of hematoxylin and eosin (H&E) and immunohistochemical (IHC) negative nodes.

85. The method of claim 83 wherein the specificity is at least 97% based on a comparison of H&E and IHC negative nodes.

86. The method of claim 83 wherein the specificity is at least 99% based on a comparison of H&E and IHC negative nodes.

87. The method of claim 83 wherein the sensitivity is at least at least 80% based on a comparison of hematoxylin and eosin (H&E) and immunohistochemical (IHC) positive nodes.

88. The method of claim 83 wherein the sensitivity is at least 85% based on a comparison of H&E and IHC positive nodes.

89. The method of claim 83 wherein the sensitivity is at least 90% based on a comparison of H&E and IHC positive nodes.

90. The method of claim 83 wherein the specificity and sensitivity are at least 97% based on a comparison of H&E and IHC negative nodes and at least 85% based on a comparison of H&E and IHC positive nodes, respectively.

91. The method of claim 73, 74, 75, 76 or 77 wherein the pre-determined cut-off levels are at least two-fold over-expression in tissue having metastatic melanoma relative to benign melanocyte or normal tissue.

92. The method of claim 73, 74, 75, 76 or 77 wherein gene expression is measured on a microarray or gene chip.

93. The method of claim 73, 74, 75, 76 or 77 wherein gene expression is determined by nucleic acid amplification conducted by polymerase chain reaction (PCR) of RNA extracted from the sample.

94.   The method of claim 93 wherein the PCR products comprise at least one of SEQ ID NOs: 25-28.

95.   The method of claim 93 wherein the PCR products include fluorophores.

96.   The method of claim 95 wherein the fluorophores are selected from the group consisting of Fam, Texas Red, Cal Red, Cl, Cy5 and Cy3.

97.   The method of claim 96 wherein the PCR product, if present, is identified by the fluorescence pattern of PLAB: Fam; L1CAM: Texas Red or Cal Red, tyrosinase: Cl; PBGD: Cy5, where applicable.

98.   The method of claim 93 wherein the PCR is reverse transcription polymerase chain reaction (RT-PCR).

99.   The method of claim 98, wherein the RT-PCR further comprises one or more internal control reagents.

100.   The method of claim 93 wherein RNA is extracted from the sample by:

   a. homogenizing the sample to produce an homogenate;

   b. contacting the homogenate with a substrate containing, or to which is affixed, an RNA-binding material;

   c. allowing the RNA to bind to the RNA binding material;

   d. washing the substrate under conditions sufficient to remove any contaminants, interferents and un-bound RNA; and

   e. eluting bound RNA from the substrate.

101.   The method of claim 73, 74, 75, 76 or 77 further comprising reducing melanin in the sample.

102.   The method of claim 101 wherein melanin concentration is reduced by homogenizing the sample to produce an homogenate and passing the homogenate through a matrix to which melanin adheres, bonds, or is affixed.

103.   The method of claim 102 wherein the matrix comprises polymeric beads.

104.   The method of claim 102 wherein the matrix comprises silica.

105.   The method of claim 100 wherein the RNA is extracted in less than about 8 minutes.

106.   The method of claim 100 wherein the RNA is extracted in less than about 6 minutes.

107.   The method of claim 73, 74, 75, 76 or 77 wherein gene expression is measured by measuring the protein encoded by the gene.

108. The method of claim 107 wherein the protein is detected by an antibody specific to the protein.

109.   A method of distinguishing a malignant melanocyte from a benign melanocyte comprising the steps of

    a. obtaining a tissue sample; and

    b. measuring the expression levels in the sample of genes recognized by the primer/probe sets selected from the group consisting of

    SEQ ID NOs: 4-6 or SEQ ID NOs: 7-9 and SEQ ID NOs: 10-12 or SEQ ID NOs: 13-15; or

    SEQ ID NOs: 4-6 or SEQ ID NOs: 7-9 and SEQ ID NOs: 10-12 or SEQ ID NOs: 13-15 and SEQ ID NOs: 16-18

    wherein the gene expression levels above pre-determined cut-off levels are indicative of the presence of a malignant melanocyte in the sample.

110.   The method of claim 109 further comprising measuring the expression level of a gene encoding tyrosinase (SEQ ID NO: 999).

111.   The method of claim 109 further comprising measuring the expression level of a gene constitutively expressed in the sample.

112.   The method of claim 110 wherein the gene encodes PBGD (SEQ ID NO: 979).

113.   The method of claim 109 further comprising measuring the expression levels of at least one gene encoding an mRNA corresponding to a psid selected from the group consisting of SEQ ID NOs: 29-978.

114.   The method of claim 109, 110, 111, 112 or 113 wherein the sample is obtained from a lymph node.

115.   The method of claim 114 wherein the lymph node is a sentinel lymph node.

116.   The method of claim 109, 110, 111, 112 or 113 wherein the sample is obtained from a biopsy.

117.   The method of claim 109, 110, 111, 112 or 113 wherein the method is performed intra-operatively.

118.   The method of claim 109, 110, 111, 112 or 113 wherein the melanoma is a micrometastasis.

119.   The method of claim 109, 110, 111, 112 or 113 wherein the specificity and sensitivity are sufficient to detect metastasis of melanoma.

120.   The method of claim 119, wherein the specificity is at least 95% based on a comparison of hematoxylin and eosin (H&E) and immunohistochemical (IHC) negative nodes.

121.   The method of claim 119 wherein the specificity is at least 97% based on a comparison of H&E and IHC negative nodes.

122.   The method of claim 119 wherein the specificity is at least *99%* based on a comparison of H&E and IHC negative nodes.

123.   The method of claim 119 wherein the sensitivity is at least at least 80% based on a comparison of hematoxylin and eosin (H&E) and immunohistochemical (IHC) positive nodes.

124.   The method of claim 119 wherein the sensitivity is at least 85% based on a comparison of H&E and IHC positive nodes.

125.   The method of claim 119 wherein the sensitivity is at least 90% based on a comparison of H&E and IHC positive nodes.

126.   The method of claim 119 wherein the specificity and sensitivity are at least 97% based on a comparison of H&E and IHC negative nodes and at least 85% based on a comparison of H&E and IHC positive nodes, respectively.

127.   The method of claim 109, 110, 111, 112 or 113 wherein the pre-determined cut-off levels are at least two-fold over-expression in tissue having metastatic melanoma relative to benign melanocyte or normal tissue.

128.   The method of claim 109, 110, 111, 112 or 113 wherein gene expression is measured on a microarray or gene chip.

129.   The method of claim 109, 110, 111, 112 or 113 wherein gene expression is determined by nucleic acid amplification conducted by polymerase chain reaction (PCR) of RNA extracted from the sample.

130.   The method of claim 129 wherein the PCR products comprise at least one of SEQ ID NOs: 25-28.

131.   The method of claim 129 wherein the PCR products include fmorophores.

132.   The method of claim 131 wherein the fluorophores are selected from the group consisting of Fam, Texas Red, Cal Red, Cl, Cy5 and Cy3.

133.   The method of claim 132 wherein the PCR product, if present, is identified by the fluorescence pattern of PLAB: Fam; L1CAM: Texas Red or Cal Red, tyrosinase: Cl; PBGD: Cy5, where applicable.

134.   The method of claim 128 wherein the PCR is reverse transcription polymerase chain reaction (RT-PCR).

135.   The method of claim 134, wherein the RT-PCR further comprises one or more internal control reagents.

136.   The method of claim 129 wherein RNA is extracted from the sample by:

    a. homogenizing the sample to produce an homogenate;

    b. contacting the homogenate with a substrate containing, or to which is affixed, an RNA-binding material;

    c. allowing the RNA to bind to the RNA binding material;

    d. washing the substrate under conditions sufficient to remove any contaminants, interferents and un-bound RNA; and

    e. eluting bound RNA from the substrate.

137.   The method of claim 109, 110, 111; 112 or 113 further comprising reducing melanin in the sample.

138.   The method of claim 136 wherein melanin concentration is reduced by homogenizing the sample to produce an homogenate and passing the homogenate through a matrix to which melanin adheres, bonds, or is affixed.

139.   The method of claim 136 wherein the matrix comprises polymeric beads.

140.   The method of claim 136 wherein the matrix comprises silica.

141.   The method of claim 136 wherein the RNA is extracted in less than about 8 minutes.

142.   The method of claim 136 wherein the RNA is extracted in less than about 6 minutes.

143. The method of claim 109, 110, 111, 112 or 113 wherein gene expression is measured by measuring the protein encoded by the gene.

144. The method of claim 143 wherein the protein is detected by an antibody specific to the protein.

145.   A method of determining patient treatment protocol comprising the steps of

    a. obtaining a tissue sample from the patient; and

b. measuring the expression levels in the sample of genes encoding

PLAB (SEQ ID NO:1) and L1CAM (SEQ ID NO:2); or

PLAB, L1CAM and NTRK3 (SEQ ID NO:3)

wherein the gene expression levels above pre-determined cut-off levels are

indicative of the presence of a melanoma in the sample.

146. The method of claim 145 further comprising measuring the expression level of a gene encoding tyrosinase (SEQ ID NO: 999).

147. The method of claim 145 further comprising measuring the expression level of a gene constitutively expressed in the sample.

148. The method of claim 147 wherein the gene encodes PBGD (SEQ ID NO: 979).

149. The method of claim 145 further comprising measuring the expression levels of at least one gene encoding an mRNA corresponds to a psid selected from the group consisting of SEQ ID NOs: 29-978.

150. The method of claim 145, 146, 147, 148 or 149 wherein the sample is obtained from a lymph node.

151. The method of claim 150 wherein the lymph node is a sentinel lymph node.

152. The method of claim 145, 146, 147, 148 or 149 wherein the sample is obtained from a biopsy.

153. The method of claim 145, 146, 147, 148 or 149 wherein the method is performed intra-operatively.

154. The method of claim 145, 146, 147, 148 or 149 wherein the melanoma is a micrometastasis .

155. The method of claim 145, 146, 147, 148 or 149 wherein the specificity and sensitivity are sufficient to detect metastasis of melanoma.

156. The method of claim 155, wherein the specificity is at least 95% based on a comparison of hematoxylin and eosin (H&E) and immunohistochemical (IHC) negative nodes.

157. The method of claim 155 wherein the specificity is at least 97% based on a comparison of H&E and IHC negative nodes.

158. The method of claim 155 wherein the specificity is at least 99% based on a comparison of H&E and IHC negative nodes.

159.   The method of claim 155 wherein the sensitivity is at least at least 80% based on a comparison of hematoxylin and eosin (H&E) and immunohistochemical (IHC) positive nodes.

160.   The method of claim 155 wherein the sensitivity is at least 85% based on a comparison of H&E and IHC positive nodes.

161.   The method of claim 155 wherein the sensitivity is at least 90% based on a comparison of H&E and IHC positive nodes.

162.   The method of claim 155 wherein the specificity and sensitivity are at least 97% based on a comparison of H&E and IHC negative nodes and at least 85% based on a comparison of H&E and IHC positive nodes, respectively.

163.   The method of claim 145, 146, 147, 148 or 149 wherein the pre-determined cut-off levels are at least two-fold over-expression in tissue having metastatic melanoma relative to benign melanocyte or normal tissue.

164.   The method of claim 145, 146, 147, 148 or 149 wherein gene expression is measured on a microarray or gene chip.

165.   The method of claim 145, 146, 147, 148 or 149 wherein gene expression is determined by nucleic acid amplification conducted by polymerase chain reaction (PCR) of RNA extracted from the sample.

166.   The method of claim 165 wherein the PCR products comprise at least one of SEQ ID NOs: 25-28.

167.   The method of claim 165 wherein the PCR products include fluorophores.

168.   The method of claim 167 wherein the fluorophores are selected from the group consisting of Fam, Texas Red, Cal Red, Cl, Cy5 and Cy3.

169.   The method of claim 168 wherein the Fluorophores correspond to PLAB: Fam; LlCAM: Texas Red or Cal Red, tyrosinase: Cl; PBGD: Cy5, where applicable.

170.   The method of claim 152 wherein the PCR is reverse transcription polymerase chain reaction (RT-PCR).

171.   The method of claim 170, wherein the RT-PCR further comprises one or more internal control reagents.

172.   The method of claim 165 wherein RNA is extracted from the sample by:

  a. homogenizing the sample to produce an homogenate;

    b.   contacting the homogenate with a substrate containing, or to which is affixed,
        an RNA-binding material;

    c.   allowing the RNA to bind to the RNA binding material;

    d.   washing the substrate under conditions sufficient to remove any contaminants,
        interferents and un-bound RNA; and

    e.   eluting bound RNA from the substrate.

173.   The method of claim 145, 146, 147, 148 or 149 further comprising reducing
melanin in the sample.

174.   The method of claim 173 wherein melanin concentration is reduced by
homogenizing the sample to produce an homogenate and passing the homogenate through
a matrix to which melanin adheres, bonds, or is affixed.

175.   The method of claim 174 wherein the matrix comprises polymeric beads.

176.   The method of claim 174 wherein the matrix comprises silica.

177.   The method of claim 172 wherein the RNA is extracted in less than about 8
minutes.

178.   The method of claim 172 wherein the RNA is extracted in less than about 6
minutes.

179. The method of claim 145, 146, 147, 148 or 149 wherein gene expression is measured
by measuring the protein encoded by the gene.   -

180. The method of claim 179 wherein the protein is detected by an antibody specific to
the protein.

181.   A method of determining patient treatment protocol comprising the steps of

    a.   obtaining a tissue sample from the patient; and

    b.   measuring the expression levels in the sample of genes identified by the
        primer/probe sets selected from the group consisting of

    SEQ ID NOs: 4-6 or SEQ ID NOs: 7-9 and SEQ ID NOs: 10-12 or SEQ ID NOs:
    13-15; or

    SEQ ID NOs: 4-6 or SEQ ID NOs: 7-9 and SEQ ID NOs: 10-12 or SEQ ID NOs:
    13-15 and SEQ ID NOs: 16-18

    wherein the gene expression levels above pre-determined cut-off levels are

    indicative of the presence of a melanoma in the sample.

182.   The method of claim 181 further comprising measuring the expression level of a gene encoding tyrosinase (SEQ ID NO: 999).

183.   The method of claim 181 further comprising measuring the expression level of a gene constitutively expressed in the sample.

184.   The method of claim 181 wherein the gene encodes PBGD (SEQ ID NO: 979).

185.   The method of claim 184 further comprising measuring the expression levels of at least one gene encoding an mRNA correspond to a psid selected from the group consisting of SEQ ID NOs: 29-978.

186.   The method of claim 181, 182, 183, 184 or 185 wherein the sample is obtained from a lymph node.

187.   The method of claim 186 wherein the lymph node is a sentinel lymph node.

188.   The method of claim 181, 182, 183, 184 or 185 wherein the sample is obtained from a biopsy.

189.   The method of claim 181, 182, 183, 184 or 185 wherein the method is performed intra-operatively.

190.   The method of claim 181, 182, 183, 184 or 185 wherein the melanoma is a micrometastasis.

191.   The method of claim 181, 182, 183, 184 or 185 wherein the specificity and sensitivity are sufficient to detect metastasis of melanoma.

192.   The method of claim 191, wherein the specificity is at least 95% based on a comparison of hematoxylin and eosin (H&E) and immunohistochemical (IHC) negative nodes.

193.   The method of claim 191 wherein the specificity is at least 97% based on a comparison of H&E and IHC negative nodes.

194.   The method of claim 191 wherein the specificity is at least 99% based on a comparison of H&E and IHC negative nodes.

195.   The method of claim 191 wherein the sensitivity is at least at least 80% based on a comparison of hematoxylin and eosin (H&E) and immunohistochemical (IHC) positive nodes.

196.   The method of claim 191 wherein the sensitivity is at least 85% based on a comparison of H&E and IHC positive nodes.

197. The method of claim 191 wherein the sensitivity is at least 90% based on a comparison of H&E and IHC positive nodes.

198. The method of claim 191 wherein the specificity and sensitivity are at least 97% based on a comparison of H&E and IHC negative nodes and at least 85% based on a comparison of H&E and IHC positive nodes, respectively.

199. The method of claim 181, 182, 183, 184 or 185 wherein the pre-determined cut-off levels are at least two-fold over-expression in tissue having metastatic melanoma relative to benign melanocyte or normal tissue.

200. The method of claim 181, 182, 183, 184 or 185 wherein gene expression is measured on a microarray or gene chip.

201. The method of claim 181, 182, 183, 184 or 185 wherein gene expression is determined by nucleic acid amplification conducted by polymerase chain reaction (PCR) of RNA extracted from the sample.

202. The method of claim 201 wherein the PCR products comprise at least one of SEQ ID NOs: 25-28.

203. The method of claim 201 wherein the PCR products include fluorophores.

204. The method of claim 203 wherein the fluorophores are selected from the group consisting of Fam, Texas Red, Cal Red, Cl, Cy5 and Cy3.

205. The method of claim 204 wherein the Fluorophores correspond to PLAB: Fam; L1CAM: Texas Red or Cal Red, tyrosinase: Cl; PBGD: Cy5, where applicable.

206. The method of claim 201 wherein the PCR is reverse transcription polymerase chain reaction (RT-PCR).

207. The method of claim 206, wherein the RT-PCR further comprises one or more internal control reagents.

208. The method of claim 201 wherein RNA is extracted from the sample by:

    a. homogenizing the sample to produce an homogenate;

    b. contacting the homogenate with a substrate containing, or to which is affixed, an RNA-binding material;

    c. allowing the RNA to bind to the RNA binding material;

    d. washing the substrate under conditions sufficient to remove any contaminants, interferents and un-bound RNA; and

    e. eluting bound RNA from the substrate.

209.   The method of claim 181, 182, 183, 184 or 185 further comprising reducing melanin in the sample.

210.   The method of claim 209 wherein melanin concentration is reduced by homogenizing the sample to produce an homogenate and passing the homogenate through a matrix to which melanin adheres, bonds, or is affixed.

211.   The method of claim 210 wherein the matrix comprises polymeric beads.

212.   The method of claim 210 wherein the matrix comprises silica.

213.   The method of claim 208 wherein the RNA is extracted in less than about 8 minutes.

214.   The method of claim 208 wherein the RNA is extracted in less than about 6 minutes.

215.   The method of claim 181, 182, 183, 184 or 185 wherein gene expression is measured by measuring the protein encoded by the gene.

216.   The method of claim 215 wherein the protein is detected by an antibody specific to the protein.

217.   A composition comprising at least one primer/probe set selected from the group consisting of: SEQ ID NOs: 4-6, SEQ ID NOs: 7-9, SEQ ID NO:46-48, SEQ ID NOs: 13-15, SEQ ID NOs: 16-18, SEQ ID NOs: 19-21, and SEQ ID NOs: 22-24.

218.   A composition comprising at least one amplicon selected from the group consisting of SEQ ID NOs: 25-28.

219.   A kit for conducting an assay to determine the presence of melanoma in a tissue sample comprising: nucleic acid amplification and detection reagents.

220.   The kit of claim 219 wherein the reagents comprise primers having sequences for detecting the expression of at least one gene encoding an mRNA selected from the group consisting of SEQ ID NOs: 1-3.

221.   The kit of claim 219 comprising RT-PCR reagents.